1: Biochim Biophys Acta 1998 Aug 14;1373 (1):119-30

Cholesterol-dependent generation of a unique amyloid beta-protein from apically missorted amyloid precursor protein in MDCK cells.

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To investigate the implications of altered sorting of the beta-amyloid precursor protein (betaAPP) in the abnormal generation of amyloid beta-protein (Abeta), we characterized Abeta secreted from Madin-Darby canine kidney (MDCK) cells which had been stably transfected with a cDNA encoding the human beta-amyloid precursor protein (betaAPP695) with a 42 amino acid residue truncation at the carboxyl terminus (DeltaC). In DeltaC MDCK cells, the intracellular sorting of betaAPP is substantially altered to the apical surface. We detected an accumulation of a unique Abeta species in the apical compartment of DeltaC MDCK cell cultures. This unique Abeta was immunoprecipitated with 4G8 (a monoclonal antibody specific for Abeta17-24) and detected as a smear on Western blots, but was not immunoprecipitated with BAN50 (a monoclonal antibody raised against Abeta1-16). Interestingly, however, this Abeta species was readily immunoprecipitated with BAN50 upon treatment with

formic acid. Furthermore, incubation of the DeltaC MDCK cells with compactin, an inhibitor of de novo cholesterol synthesis, or with filipin, a cholesterol-binding drug, resulted in marked changes in the characteristics of this Abeta species as follows: first, the Abeta was not observed as a smear on Western blots and second, the Abeta was immunoprecipitated with BAN50. The present results strongly suggest that an Abeta with unique molecular characteristics is generated from the missorted betaAPP in vivo in a cholesterol-dependent manner.

PMID: 9733943 [PubMed - indexed for MEDLINE]

1: Biochem Biophys Res Commun 1996 Mar 27;220(3):710-8

Nuclear and cytoplasmic localization of the beta-amyloid peptide (1-43) in transfected 293 cells.

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Cultures of transformed human embryonic kidney 293 cells were transiently transfected with minigene constructs coding for the Abeta peptide (1-43). The Abeta minigene used in this study consisted of exons 16 and 17 of the amyloid precursor protein gene, including the 6000+ bp intronic region. Two of the constructs used in this study, human amyloid precursor protein (APP) promoter-driven Abeta minigene and BK virus enhancer/adenovirus major late promoter-driven Abeta minigene, did not contain a signal peptide sequence, whereas the third, human APP promoter-signal peptide Abeta minigene did not contain the human APP signal sequence. The resulting Abeta products were detected by immune precipitation, using 10D5 antibody and Western blot analysis, using R1280 antisera, as SDS stable oligomers in cell lysates of cells containing all three constructs or in culture media when produced by the signal peptide construct. Evaluation of the cells by immunocytochemistry using conventional and

transmission electron microscopy indicated that the cells transfected with constructs without the signal peptide accumulated immunoreactive Abeta primarily in the nucleus.

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1: Biochemistry 1998 Oct 20;37 (42):14958-65

Processing of the Alzheimer's disease amyloid precursor protein in Pichia pastoris: immunodetection of alpha-, beta-, and gamma-secretase products.

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betaA4 (Abeta) amyloid peptide, a major component of Alzheimer's disease (AD) plaques, is a proteolytic product of the amyloid precursor protein (APP). Endoproteases, termed beta- and gamma-secretase, release respectively the N- and C-termini of the peptide. APP default secretion involves cleavage within the betaA4 domain by alpha-secretase. To study the conservation of APP processing in lower eukaryotes, the yeast Pichia pastoris was transfected with human APP695 cDNA. In addition to the full-length integral transmembrane protein found in the cell lysate, soluble/secreted APP (sAPP) was detected in the culture medium. Most sAPP comprised the N-terminal moiety of betaA4 and corresponds to sAPPalpha, the product of alpha-secretase. The culture medium also contained minor secreted forms detected by a monoclonal antibody specific for sAPPbeta (the ectodomain released by beta-secretase

cleavage). Analysis of the cell lysates with specific antibodies also detected membrane-associated C-terminal fragments corresponding to the products of alpha and beta cleavages. Moreover, immunoprecipitation of the culture medium with three antibodies directed at distinct epitopes of the betaA4 domain yielded a 4 kDa product with the same electrophoretic mobility as betaA4 synthetic peptide. These results suggest that the alpha-, beta-, and gamma-secretase cleavages are conserved in yeast and that P. pastoris may offer an alternative to mammalian cells to identify the proteases involved in the generation of AD betaA4 amyloid.

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Immunohistochemical localization of amyloid beta-protein with amino-terminal aspartate in the cerebral cortex of patients with Alzheimer's disease.

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We investigated immunohistochemically the localization of amyloid beta-protein (Abeta) with amino-terminal aspartate (N1[D]) in brains of patients with Alzheimer's disease, diffuse Lewy body disease and Down's syndrome. A monoclonal antibody, 4G8, which recognizes the middle portion of Abeta, was used as a reference antibody to label the total Abeta deposits. Double staining with anti-Abeta (N1[D]) and 4G8 revealed that Abeta deposits in the subiculum and the neocortical deep layers often lacked N1 [D] immunoreactivity, indicating N-terminal truncation of Abeta in these deposits. Abeta deposits in the neocortical superficial layers and the presubicular parvopyramidal layer always contained Abeta with N1[D]. Such regional as well as laminar differences in the distribution of Abeta beginning at N1[D] suggest that some local factors influence N-terminal processing of Abeta deposited in the brain.

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Original Article

Occurrence of the diffuse amyloid β-protein (Aβ) deposits with numerous Aβ-containing glial cells in the cerebral cortex of patients with Alzheimer's disease

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Aβ40; microglia; astrocytes; clearance; uptake; degradation

Abstract

Diffuse amyloid β-protein (Aβ) deposits with numerous glial cells containing C-terminal Aβ fragments occur in the cerebral cortex of patients with Alzheimer's disease. By using a panel of antibodies specific for various epitopes in the Aβ peptide, we have investigated the immunohistochemical nature of the diffuse Aβ deposits. The extracellular material contains Aβ with a C-terminus at residue valine^{40} (Aβ40) as well as residues alanine^{42}/threonine^{43} (Aβ42). The N-termini include aspartate^{1}, pyroglutamate^{3}, and pyroglutamate^{11}, with pyroglutamate^{3} being dominant. Microglia and astrocytes in and around these deposits contain intensely staining granules. Most of these granules are negative for antibodies to the N-terminally located sequences of Aβ. These include 6E10 (Aβ1-17), 6F/3D (Aβ8-17), and the N-terminal antibodies specific to aspartate^{1}, pyroglutamate^{3}, and pyroglutamate^{11}. The C-termini of intraglial Aβ are comparable with those of the extracellular deposits. The microglia and astrocytes have quiescent morphology compared with those associated with senile plaques and other lesions such as ischemia. Complement activation in these deposits is not prominent and often below the sensitivity of immunohistochemical detection. Although factors which may cause this type of deposit remain unclear, lack of strong tissue responses suggests that these deposits are a very early stage of Aβ deposition. They were found only inconsistently and were absent in a number of cases examined in this study. Further analysis of these deposits might provide important clues regarding the accumulation and clearance of Aβ in Alzheimer's disease brain. GLIA 25:324-331, 1999. © 1999 Wiley-Liss, Inc.

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